



Ion AmpliSeq Direct FFPE DNA Kit

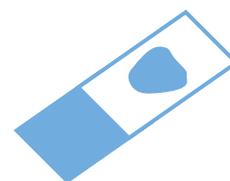
A new direction in FFPE DNA preparation

Maximum recovery from every precious sample

Analysis of DNA from formalin-fixed, paraffin-embedded (FFPE) samples can be challenging, as both quality and yield vary widely according to tissue procurement, processing, and storage conditions. Traditional methods to extract and purify DNA from FFPE samples, which require both deparaffinization and purification procedures, can further hinder analysis, as the unavoidable sample loss can reduce the overall yield of DNA to a level that is too low for molecular analysis. Targeted sequencing is possible for more of your precious FFPE samples using the new Ion AmpliSeq™ Direct FFPE DNA Kit. In only 30 minutes, FFPE DNA is ready for Ion AmpliSeq™ library prep, helping you to extract the maximum information from more FFPE samples.

Ion AmpliSeq Direct FFPE DNA enables:

- **Fast sample-to-answer**—High-quality FFPE DNA is prepared in only 30 minutes with just 2 pipetting steps and <10 minutes hands-on time, from a tissue slice of 0.25 cm² or less.
- **Maximum recovery**—Loss of DNA is minimized via a single-tube, two-step protocol combined with the use of Direct Reagent, which eliminates the requirement for deparaffinization procedures and subsequent column or bead purification steps.
- **Targeted sequencing success**—Ion AmpliSeq technology accommodates a wide input range of FFPE DNA, from 1–100 ng.* Either quantify DNA or input maximum sample volume into library prep. Maximum recovery of DNA combined with low-input DNA requirements for Ion AmpliSeq targeted sequencing means that more genetic queries can be answered from each FFPE sample. Optional UDG protocol is designed to remove deaminated cytosines for variant analysis.



Sample



Step 1

Transfer sample to tube or plate



Step 2

Add Direct Reagent and incubate for 15 min at 65°C

Ready-to-use DNA

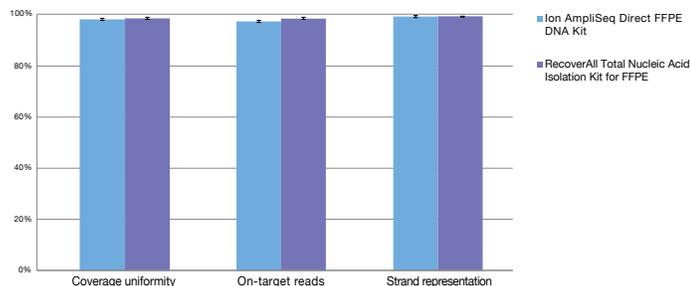


Figure 1. Ion AmpliSeq Direct FFPE DNA has comparable performance to purified DNA. The Ion AmpliSeq Direct FFPE DNA Kit or the Invitrogen™ RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE were used to prepare DNA from FFPE lung research samples (n=4). Libraries were created using the Ion AmpliSeq™ Cancer Hotspot Panel v2, prepared using the Ion Chef System, and sequenced using the Ion S5 System. Data was analyzed using Torrent Suite Software. The data illustrates comparable performance for both methods of DNA preparation, with coverage uniformity[†] of >98%, on-target reads[‡] >97%, and strand bias <1%.

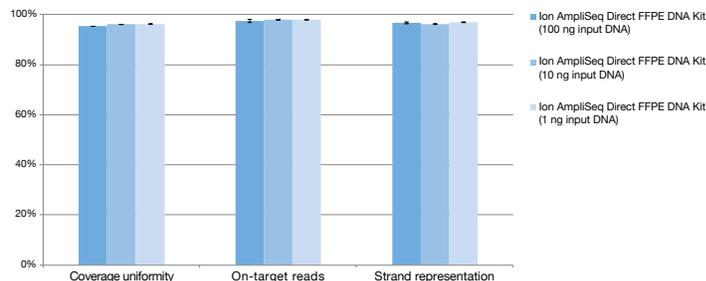


Figure 2. Comparable targeted sequencing performance for 1-100 ng of input DNA. DNA was prepared from 1 cm² sections of FFPE liver research samples (n=2) using the Ion AmpliSeq Direct FFPE DNA Kit protocol and then quantified using the Invitrogen™ Qubit™ dsDNA HS Assay Kit. Input DNA amounts of 1 ng, 10 ng or 100 ng were used to generate targeted Ion AmpliSeq libraries,** prepared using the Ion AmpliSeq™ Kit for Ion Chef DL8, sequenced using the Ion PGM™ System, and analyzed using Torrent Suite™ Software. Comparable performance was demonstrated for all samples with coverage uniformity[†] >95%, on-target reads[‡] >97%, and strand bias <5%.

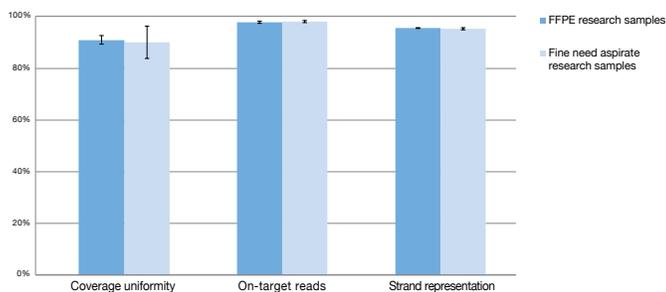


Figure 3. Direct FFPE DNA Kit can be used with fine needle aspirate samples. The Ion AmpliSeq Direct FFPE DNA Kit protocol was used to prepare DNA from FFPE liver and fine needle aspirate research samples (n=4). Libraries were created using an Ion AmpliSeq gene panel,** prepared using the Ion OneTouch™ 2 System, and sequenced on the Ion PGM System. Data was analyzed using Torrent Suite Software. The data illustrates comparable performance of both FFPE and fine needle aspirate research samples with coverage uniformity[†] of >90%, on-target reads[‡] >97%, and strand bias <5%.

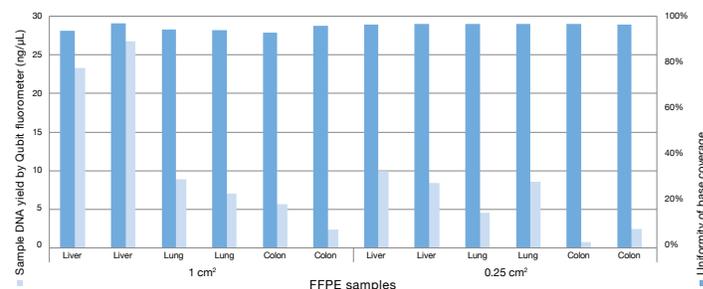


Figure 4. Robust targeted sequencing for different tissue types. Liver, lung, and colon FFPE sections (n=4) of either 0.25 cm² or 1 cm² were prepared using the Ion AmpliSeq Direct FFPE DNA Kit protocol and quantified using the Qubit dsDNA HS Assay Kit. Libraries were created using an Ion AmpliSeq gene panel,** and sequenced using the Ion S5™ System. Data was analyzed using Torrent Suite Software. High-quality libraries were generated with uniformity of base coverage >92% for all tissue types shown.

Ordering information

Product	Cat. No.
Ion AmpliSeq Direct FFPE DNA Kit (8 reactions)	A31133
Ion AmpliSeq Direct FFPE DNA Kit (96 reactions)	A31136

Learn more at thermofisher.com/directffpedna

* Reproducible results were observed from the manufacturer's verification studies whereby 0.25 cm² FFPE DNA starting material was used for analysis, and when following the manufacturer's recommended protocol. Reproducible results were also reported by external test sites using less than 0.25 cm² of FFPE starting material. Your observed results may be different, as the quantity and quality of FFPE DNA can vary significantly from sample to sample.

** An Ion AmpliSeq high-content gene panel, targeting 134 genes and containing 2,530 primer pairs, was used to demonstrate targeted sequencing performance.

[†] Coverage uniformity = percentage of bases covered at ≥20% of the mean coverage.

[‡] On-target reads = percentage of reads that mapped to target regions, out of total mapped reads per run.

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